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A one-day test for determining frost hardiness using the electrical conductivity technique

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Introduction

In Ontario, frost hardiness testing is almost without exception the basis for determining when containerized tree seedlings in greenhouses are hardy enough to be moved outside for overwintering. In the electrical conductivity technique described earlier (Colombo et al. 1984), hardiness is determined following a controlled freezing test of seedling shoot tips to a minimum temperature of -15°C. Freezing disrupts cell membranes, which allows leakage of the cell's electrolytic contents. Freezing damage can therefore be determined by immersing the shoots in water; the increase in the solution conductivity will be proportional to the amount of damage (Colombo et al. 1982).

Using the standard electrical conductivity procedure for measuring frost hardiness, test results are obtained in three days. This is a constraint upon operations, since approximately 45 million container seedlings from nearly 30 nurseries are annually overwintered in Ontario. Testing is conducted in laboratories, currently located at the Ontario Tree Improvement and Forest Biomass Institute, and the Northern and Northwestern Regions, and similar facilities have now also been established in New Brunswick, Manitoba and Alberta. We have developed a technique which shortens the time required for measuring the degree of damage from three days to one day. This note describes this revised procedure.

Description of the Standard Procedure

In the electrical conductivity technique currently in operational use and which was previously described by Colombo et al. (1984), a total of 45 black spruce shoot tips or 21 shoot tips for jack pine are collected on a weekly basis from each sampling unit (usually each greenhouse or seedlot within a greenhouse). The shoot tips are divided into 3 groups of equal number and are placed in sample jars. One group serves as a non-frozen control, to which deionized or distilled water is immediately added in the sample jars. Between 16 and 24 hours later the electrical conductivity of the control solution is measured (EC_c). The remaining 2 groups are frozen at a rate of 5°C per hour to -15°C and thawed (usually overnight) before water is added. Sixteen to 24 hours later the electrical conductivity of the solution of frozen samples is measured (EC_f). Following measurement of initial conductivity values (EC_c or EC_f) the samples are killed by being placed in a 90°C oven for 4 hours. Electrical conductivities are remeasured

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16 to 24 hours later to obtain EC_k values. In total, 3 days elapse from the time samples are prepared for freezing until killed conductivities are measured in the standard procedure.

Frost hardiness is determined with the electrical conductivity technique using an Index of Injury (I_t), calculated according to Flint et al. (1967) by:

$$I_t = \frac{\frac{EC_f}{EC_k} - \frac{EC_c}{EC_k}}{1 - \frac{EC_c}{EC_k}} \times 100 \qquad [1]$$

Description of the One-Day Test

Shortening the electrical conductivity technique from three days to a one day test relies upon the measurement of the electrical conductivity of a sample of killed shoot tips (EC_k), determined once at the beginning of the test period. A separate measurement of EC_k should be obtained for seedlings from each greenhouse and seedlot to be tested. This average EC_k, adjusted to a standard solution volume/shoot tip weight basis (Appendix A), is used in all subsequent calculations of Index of Injury, combined with EC_f values calculated each week. The test requires only 24 hours when performed as follows:

Day 1
1/ Collect and prepare samples for freezing as described elsewhere (Colombo et al. 1984), except that the solution volume and shoot tip weight of each sample must be measured.
2/ Freeze samples at a rate of 5°C per hour to -15°C.
3/ Thaw samples at a rate of 20°C per hour to above freezing.
4/ Add distilled/deionized water to thawed samples. Leave overnight.

Day 2
1/ Measure EC's and calculate Index of Injury using average EC_k (Appendix B) and adjusted EC values.

Comparison of One-Day and Standard Test Results

Correlations between Index of Injury values determined using the Standard and the One-Day procedures are shown in figure 1a and 1b for jack pine and black spruce respectively. Values were obtained from the North Central Region's 1986 operationally monitored crops. Index of Injury values were obtained over the course of hardening, from bud initiation to hardening to -15°C for both species. The correlations between the Standard and One-Day procedures were highly significant ($p < 0.01$), with r^2's of 0.950 for jack pine and 0.963 for black spruce.

Conclusions

Container seedlings are considered sufficiently hardy to be moved outside when Index of Injury of the shoot tips has been 5% or less for two consecutive tests. This criterion is almost always adequate to prevent shoot freezing damage to seedlings which are moved outside in northern Ontario no later than the first week of November. The short test procedure reported here allows test results to be obtained more quickly, permitting seedlings to be moved outside earlier and facilitating the measurement of larger numbers of samples.

This short test procedure will yield results within 24 hours of the start of the freezing test (using a minimum temperature of -15°C) if samples are thawed at a rate of approximately 20°C per hour following freezing.

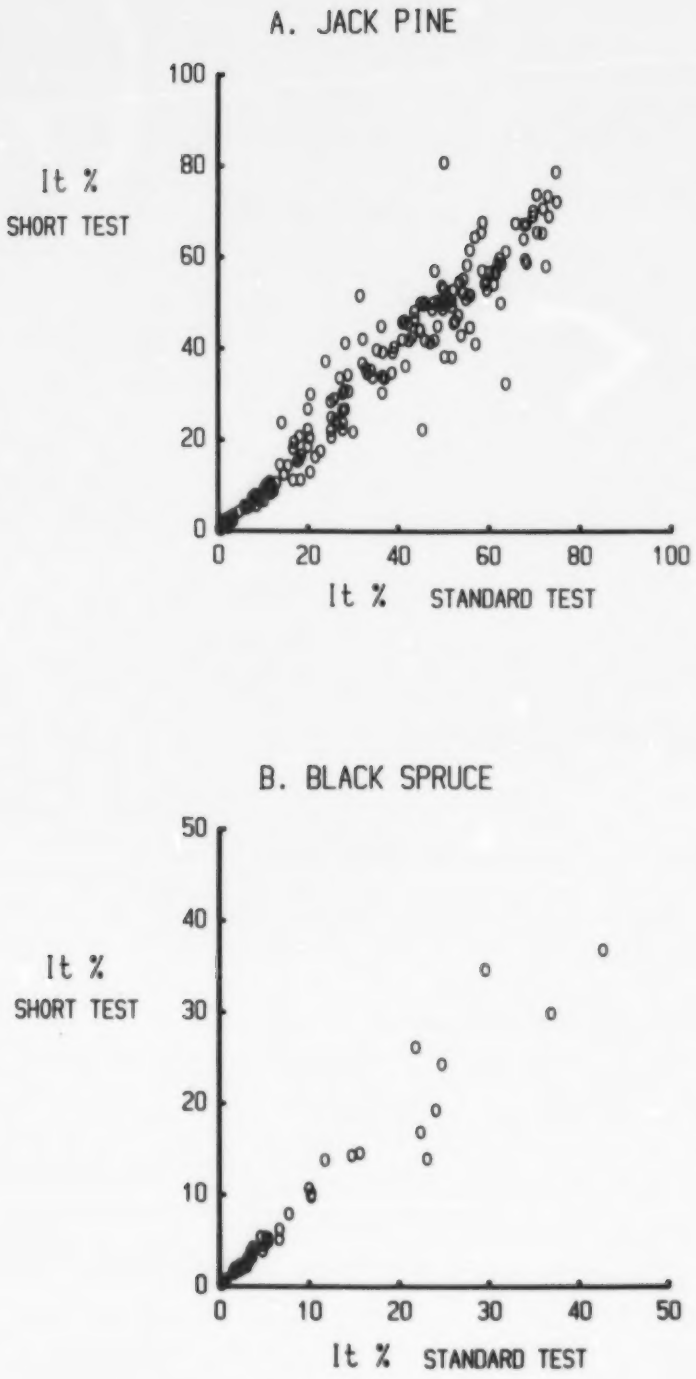

Figure 1 Correlations between Index of Injury measured using standard and revised procedures for A. Jack pine (n=237) and B. Black spruce (n=141).

Acceptable rates of thawing can usually be achieved by placing the uncapped sample bottles on a laboratory bench for 2-4 hours prior to adding water. Caution, however, must be taken to ensure that shoot tips are thawed when water is added, as water added directly to frozen shoot tips will result in almost instantaneous thawing, which can itself cause damage and therefore give an incorrectly large EC_f and a subsequent under-estimation of frost hardiness.

References

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APPENDIX A. Procedure for adjusting measured EC values to a common solution volume/shoot tip weight basis.

1/ Determine total shoot tip weight (mg) and solution volume (ml) for the sample whose EC value is to be adjusted.
2/ Adjust EC values using the following formula:

$$\text{Adjusted EC} = \frac{\dfrac{\text{measured solution volume}}{\text{measured shoot tip weight}} \times \text{measured EC}}{0.025}$$

APPENDIX B. Procedures for estimating average EC_k for use in the one-day electrical conductivity technique.

1/ Collect 90 shoot tips from each seedlot/greenhouse.
2/ Place the shoot tips into 6 groups of 15.
3/ Weigh each group of shoot tips to the nearest milligram. Care should be taken to avoid desiccation of the samples.
4/ Place 15 shoot tips into 6 test bottles and add a known volume of deionized or distilled water to each bottle, sufficient in volume to completely submerge the shoot tips. Cap the bottles.
5/ Kill the shoot tips by placing the bottles in a 90°C oven for 4 hours.
6/ Incubate the samples at room temperature for at least 16 hours but not more than 24 hours and measure electrical conductivity of the solution (temperature corrected).
7/ Adjust EC_k readings to a 0.025 solution volume/shoot tip weight ratio (see Appendix A).
8/ Calculate average adjusted EC_k of the 6 bottles for each seedlot/greenhouse. Following calculation of this average EC_k, Index of Injury can be determined following standard procedures.

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